# Epstein–Barr virus and multiple sclerosis

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## Purpose of review

Recent studies have revived interest in the long-scrutinized association between Epstein–Barr virus (EBV) and multiple sclerosis (MS). We review this evidence and discuss it in relation to MS pathological and clinical features and patients' response to immunosuppressive therapies.

## Recent findings

Serological evidence of previous exposure to EBV in children with MS supports a role for EBV infection early in MS pathogenesis, as already indicated by prospective studies in adults. Higher antibody titers and T-cell responses to EBV in patients compared to healthy EBV carriers indicate possible continuous viral reactivation, whereas there is some evidence that EBV could break immune tolerance to myelin antigens through molecular mimicry. Detection of EBV-infected B-cells in patients' brain raises the possibility that intrathecal B-cell abnormalities and T-cell-mediated immunopathology in MS are the consequence of a persistently dysregulated EBV infection. Accordingly, targeting T-cells and/or B-cells with monoclonal antibody therapies ameliorates MS. Whether EBV has a causative or pathogenic role in MS can now be addressed in relation to genetic, hormonal and other environmental influences that may affect EBV–host interactions.

## Summary

By shedding light on the involvement of EBV in MS, these findings will pave the way to disease prevention and increase the therapeutic index of future treatments.

## Keywords

B-cells, Epstein–Barr virus, multiple sclerosis

## Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by intrathecal production of antibodies with largely unknown specificity and by inflammatory damage to myelin and neurons. Both genetic and environmental factors contribute to the cause of MS but we do not understand yet how these factors interact to initiate the disease [1]. That MS is most likely a rare consequence of a common infection was postulated several decades ago, largely on the basis of its geographical distribution and change in risk among migrants. Among infectious agents, Epstein–Barr virus (EBV) shows a strong association with MS and other common autoimmune diseases on the basis of epidemiological and serological evidence [2]. EBV is a B-lymphotropic herpesvirus that infects 90–95% of the world population and can cause infectious mononucleosis. Following primary infection, EBV usually establishes a life-long, asymptomatic infection in B-cells which is maintained through expression of a limited set of viral latency genes and a low level of viral production in lymphoid tissue [3]. EBV infection is normally held in check by the immune system but may reactivate in individuals with genetic and acquired immunodeficiencies or undergoing immunosuppression. Here, we review latest work addressing the role of EBV infection in MS and critically discuss elements of compatibility between EBV biology and MS pathological and clinical features.

## Immune response to Epstein–Barr virus

There is consensus that adult MS patients are all EBV-seropositive and have higher titers of anti-EBV antibodies than healthy virus carriers [2,4]. A previous history of infectious mononucleosis and elevation of antibody titers to EBV antigens [particularly EBV nuclear antigen 1 (EBNA1)] are associated with an increased risk of developing MS, suggesting a role for EBV early in MS pathogenesis [4]. Accumulating evidence indicates that EBV seroprevalence and anti-EBV serum titers are also higher in children with MS as compared with age-matched controls, whereas no differences are noted for other common childhood viruses [5–7]. Although the

study of pediatric cohorts confirms early dysregulation of EBV infection in MS development, the observation that the EBV seroprevalence rate in pediatric MS is around 90% [5–7] would argue against the association between EBV and MS being causative. However, as it is more difficult to diagnose MS in children, long-term follow-up of EBV-seronegative childhood MS cases will be necessary to assess whether they turn out to have MS or not.

Consistent with continuous EBV reactivation, there is accumulating evidence of altered T-cell reactivity to EBV in MS. Compared to control patients, MS patients have increased CD4+ and CD8+ T-cell responses to EBV antigens, particularly EBNA1 [8,9,10•]. EBNA1-specific CD4+ T-cells from MS patients partially cross-react with myelin antigens [11•], supporting earlier findings based on the fine specificity of T-cell clones [12] and the possibility that EBV infection triggers CNS autoimmunity through molecular mimicry. Jilek and colleagues [10•] found higher CD8+ T-cell responses to EBV lysates or viral peptides in patients with a clinically isolated syndrome than in patients with relapsing–remitting and progressive MS. In apparent contrast with these studies, Pender and colleagues [13•] reported decreased CD8+ T-cell responses to EBV-infected lymphoblastoid cell lines in MS patients with a mean disease duration of $10.1 \pm 7.6$ years as compared to controls, suggesting that a defect in the immune control of EBV may play a role in MS. Longitudinal studies need to be performed to learn more on the frequency and functional status of EBV-specific T-cells in relation to disease duration, phase of disease and brain lesion activity. This type of analysis may allow us to understand whether perturbed EBV infection in MS leads to exhaustion of the virus-specific immune response with an impact on the clinical outcome. T-cell dysfunction with lack of viral control is a characteristic feature of chronic infections, probably aimed at preventing overt immunopathology but perhaps detrimental to optimal antiviral immunity [14].

## Neuropathological findings
In MS, multifocal, demyelinated lesions tend to accumulate in the periventricular white matter and at the surface of the brain and spinal cord. Brain-infiltrating inflammatory cells (mainly T-cells, B-cells, plasma cells and macrophages) localize in the perivascular spaces of the white matter and in the meninges. The latter can harbor large B-cell aggregates resembling lymphoid follicles with germinal centers [15]. A recent analysis of postmortem brain tissue from MS patients has revealed that many of the CNS-infiltrating B-cells express markers of latent EBV infection and that EBV reactivates in plasma cells in acute MS lesions and ectopic B-cell follicles [16]. Although waiting for independent replication, these findings establish a direct link between altered immune

reactivity to EBV and inflammatory brain disease in MS. The increased EBV load in the CNS [16], but not in the blood [9,17], of MS patients raises the possibility that a locally dysregulated viral infection may trigger an immunopathological response causing brain tissue damage. Given that CD8+ T-cells have a key role in the control of EBV infection [18], the preferential accumulation and clonal expansion of CD8+ T-cells in MS lesions [19,20], coupled with evidence of cytotoxicity toward intracerebral EBV-infected plasma cells [16] and increased CD8+ T-cell-mediated cytotoxic activity in the cerebrospinal fluid (CSF) during MS relapses [21], are consistent with an ongoing antiviral immune response in the MS brain.

Even though the characteristics of the brain immune infiltrate in MS are compatible with an immunopathological response caused by dysregulated EBV infection, questions remain as to which are the early events that allow migration and survival of infected B-cells in the brain and whether an autoimmune component contributes to inflammatory brain damage. Elegant studies in animal models have shown that in diseases driven by viruses prone to establish chronic infections, T-cell hyperreactivity delays the production of virus-neutralizing antibodies and allows the establishment of 'extra-lymphatic viral sanctuaries' that show features reminiscent of autoimmune responses [22]. It can be envisaged that any persistent or transient event affecting anti-EBV immunity, including infection with unique viral genotypes, may increase the probability that the brain turns into an 'EBV sanctuary' in which the immune system is unable to completely clear the virus and loss of immune tolerance toward self antigens may be facilitated.

## Intrathecal immunoglobulin synthesis and oligoclonal IgG
More than 90% of patients with MS have high concentrations of immunoglobulin (Ig), mainly oligoclonal IgG, in the CSF and brain tissue whose synthesis is sustained intrathecally. Oligoclonal IgG in the CSF are typically found in CNS infections, bind with high affinity to the causative pathogen, and may disappear after the infection is cleared. In contrast, in MS oligoclonal bands are a persistent phenomenon and their specificity remains largely unknown [15]. By analogy with CNS infections, the presence of oligoclonal IgG binding to EBV antigens in the CSF of some MS patients would support a role for EBV in causing MS [8,16]. Polyspecific immunoglobulins recognizing several common pathogens are also synthesized in the CNS of most MS patients and are viewed as a bystander reaction of an ongoing immune response [15].

B-cells isolated from MS brain lesions and CSF show features of an antigen-driven process that could be

supported within ectopic B-cell follicles in the inflamed CNS [15]. However, the detection of EBV-infected B-cells in the MS brain raises the possibility that intrathecal B-cell activation is the direct consequence of EBV-driven polyclonal and oligoclonal activation, rather than of chronic antigenic stimulation. Through latency proteins EBV mimics the signals that are normally delivered by antigen and T-cells to drive B-cell expansion, survival and maturation [3]. EBV latency proteins are highly expressed in the MS brain [16] and could promote the survival of EBV-infected memory B-cells that have migrated from the blood circulation leading to intrathecal production of antibodies with heterogeneous specificities [15]. The fact that CSF oligoclonal IgG is not abolished even after immunoablation [23] indicates that the CNS harbors an extremely resistant population of activated B-cells whose persistence may be congruent both with a local viral infection and with the B-cell-activating properties of EBV.

## Disease course and brain inflammatory activity

In 80–85% of patients, MS begins as a relapsing episodic disorder (relapsing–remitting MS) that evolves later into a progressive phase characterized by accumulation of neurologic disability (secondary progressive MS). Less frequently, progression of disability can manifest at the beginning of the disease (primary progressive MS). Focal brain inflammatory activity, as assessed by gadolinium-enhanced magnetic resonance imaging, is prominent at early disease stages and slows down during disease progression. Periodic reactivation, probably elicited by stress events such as exposure to other infectious agents, is a characteristic feature of herpesviruses that establish a latent infection. It is therefore tempting to juxtapose the relapsing–remitting course of MS and recurrent EBV reactivation driving a detrimental immunological response, although it may prove difficult to distinguish between EBV reactivation exacerbating MS inflammation or another underlying inflammatory event leading to both MS flare and EBV reactivation. Evidence that EBV reactivation associates with brain lesion activity or clinical relapse is still scanty and not unequivocal [17,24–26]. Nonetheless, detection of viral lytic cycle proteins in acute MS lesions suggests a relationship between EBV reactivation and brain inflammation [16]. To clarify this issue, longitudinal magnetic resonance imaging studies need to be performed in parallel with analysis of EBV-specific humoral and cellular immune responses. Consistent with EBV reactivation in brain niches of viral persistence is also the observation that most clinical relapses in MS tend to recur at sites in which inflammation has already occurred in the past [27].

## Reduced relapse rate during pregnancy

In women with MS, the relapse rate is reduced during pregnancy and is increased in the first trimester postpartum. Notably, reactivation of EBV, but not of other ubiquitous latent viruses, is observed in healthy pregnant women more frequently than in control patients, as evidenced by higher titers of antibodies to EBV early antigen and viral capsid antigen (VCA) [28]. The decreased frequency of MS relapses during gestation, when EBV infection is prone to reactivation, could be explained by the state of partial immune suppression that characterizes pregnancy [29]. As a consequence, if intracerebral EBV reactivation occurred during this period it would not result in the recruitment and activation of cytotoxic lymphocytes in the CNS with subsequent inflammation. However, when the immune system recovers to nonpregnant levels during the puerperium, it may bolster the inflammatory response in the CNS in the attempt to clear foci of EBV reactivation. This scenario would be consistent with the increased risk of relapses in the puerperium.

## Genetic risk factors

Irrespective of their weight, the major genetic associations identified to date support a defect of immune regulation in MS. The strongest genetic associations have been confirmed for alleles of the human leukocyte antigen (HLA) class II region but an influence of certain HLA class I alleles on MS risk is increasingly recognized [30]. Since HLA class I and II molecules determine the efficiency of the presentation of viral peptides to CD8+ and CD4+ T-cells, respectively, it is plausible that allelic variants of their encoding genes may affect the immune response to EBV leading to suboptimal control of viral infection. Although both latent and lytic infection are under the control of CD8+ cytotoxic T-cells, CD4+ T-cell responses also play a role in controlling EBV infection [18]. Recent studies have demonstrated an association between certain HLA class I alleles and the risk of developing infectious mononucleosis [31]. The same HLA class I alleles have been linked to EBV-positive Hodgkin's lymphoma. Interestingly, Habek and colleagues [32] reported the case of a male patient who developed in sequence infectious mononucleosis, Hodgkin's disease and MS. Because a previous history of infectious mononucleosis increases the risk of MS by 2–3-fold, it will be important to investigate whether infectious mononucleosis and MS share common susceptibility or protection genes. Recently, allelic variants of the interleukin (IL)-2 receptor α [33] and IL-7 receptor α ([33–35] genes have been identified which explain a small proportion of the MS risk but could play a role in the regulation of the immune response to EBV. A new association that may establish a more direct connection between MS and EBV is the one involving a genetic

variant of perforin, a gene that has been implicated in familial hemophagocytic lymphohistiocytosis, an EBV-associated disease [36•].

A few studies have started to address the interaction between EBV, MS susceptibility genes and other MS risk factors. In healthy individuals, anti-VCA IgG levels were found to correlate with female sex, HLA-DR2 and tobacco smoking [37], suggesting that these major predisposing factors may be linked to MS via a dysregulated immune response to EBV. Recently, other studies found that anti-EBNA1 antibody titers are a risk factor for MS independently from the DRB1∗1501 allele [38,39] and that the MS risk conferred by HLA-DRB1∗15 is higher in

**Table 1 Is EBV 'MS-compatible'?**

| Observations in MS patients | Compatibility with EBV | Refs |
| --- | --- | --- |
| Epidemiological features: socioeconomic influence, latitude gradient, earlier onset in women than in men, higher risk to develop MS after late EBV infection | Similarities between the epidemiology of infectious mononucleosis and that of MS | [2,4] |
| Serological evidence of remote EBV infection in 100% of adult MS patients and about 90% of children with MS; increased risk of developing MS in individuals with elevated anti-EBNA1 antibodies and prior infectious mononucleosis | Previous exposure to the virus required, but not sufficient, to develop MS | [2,4–7] |
| Increased immune reactivity to EBV in MS patients compared to controls: higher serum levels of antibodies to EBNA1 and, in a subset of patients, early antigens; presence of oligoclonal antibodies binding EBV antigens in the CSF; increased EBV-specific CD4+ and/or CD8+ T-cell responses; lower EBV-specific CD8+ T-cell responses | Persistent viral reactivation | [4,8,9,10•,11•, 13•,16,24] |
| Neuropathological features: presence of focal lesions in the brain and spinal cord white matter and predominance of subpial lesions in the cerebral grey matter; predominance of CD8+ T-cells and CD8+ T-cell clonal expansions in brain lesions and CSF | Both EBV latent and lytic antigens elicit a strong and potentially detrimental cytotoxic CD8+ T-cell response. Homing and expansion of EBV-infected B-cells in the perivascular spaces of blood vessels in the white matter and in the subarachnoid space would result in the recruitment of cytotoxic lymphocytes and bystander brain tissue damage | [16,18–20] |
| Abnormal B-cell activation in the CNS: intrathecal antibody synthesis; presence of oligoclonal IgG; accumulation of clonally expanded and antigen-experienced B-cells and plasma cells; formation of ectopic B-cell follicles; B-cell proliferation in ectopic B-cell follicles and acute MS lesions | Activation, expansion and maturation of EBV-infected B-cells is stimulated by viral latency proteins. EBNA2 drives naïve B-cells into proliferation; latent membrane protein (LMP)1 and LMP2A promote B-cell proliferation, survival and maturation by acting as constitutive, ligand-independent mimics of CD40 and B-cell receptor, respectively. These EBV-driven signals may induce B-cell activation in the absence of antigenic stimuli and T-cell help | [3,15,16] |
| MS relapses and shift to secondary progressive phase | Persistent EBV reactivation leading to exhaustion of the antiviral immune response | [14] |
| Reduced clinical disease activity during pregnancy | Reduced anti-EBV immune response due to the state of relative immune suppression, as inferred from increased EBV reactivation during pregnancy | [28,29] |
| Increased clinical disease activity during the puerperium | Restoration of optimal immune surveillance toward EBV | [28] |
| Increased risk of developing MS conferred by HLA-DR alleles and, to a lesser extent, by HLA class I alleles and variants of IL-2 receptor α, IL-7 receptor α and perforin genes | Dysregulation of EBV infection consequent to suboptimal immune control | [30,33–35,36•] |
| Beneficial effect of type 1 interferon on disease activity | Potentiation of antiviral immunity | |
| Beneficial effect of immune suppression (e.g. corticosteroids, chemotherapeutics) on disease activity | Inhibition of EBV-specific immune responses resulting in the reduction of immunopathological brain tissue damage | |
| Beneficial effect of monoclonal antibody therapies on disease activity | Elimination/reduction of circulating EBV-specific T-cells (alemtuzumab, fingolimod) or inhibition of leukocyte migration into the CNS (natalizumab); elimination/reduction of EBV-infected B cells (rituximab) | [41–43,44••] |

CNS, central nervous system; CSF, cerebrospinal fluid; EBNA1, EBV nuclear antigen 1; EBV, Epstein–Barr virus; HLA, human leukocyte antigen; MS, multiple sclerosis.

the presence than in the absence of infectious mononucleosis [40].

## Beneficial effects of immunosuppressive therapies

Whereas the partial efficacy of an immunomodulatory and antiviral agent such as interferon-β is compatible with the involvement of EBV in MS pathogenesis, with the advent of new biological treatments it is becoming increasingly clear that the stronger the immune suppression, the higher the apparent efficacy (particularly on the relapse rate in relapsing–remitting MS). At first sight, this evidence may be against the involvement of an infectious agent in the disease. Nonetheless, in conditions driven by viruses that are prone to establish persistent infections, including EBV, overactive and dysregulated immune responses are a risk factor for viral persistence and severe disease [14,18,22]. Whatever the mechanism underlying the efficacy of biologic therapeutics approved or under study in MS [blockade of leukocyte entry into the CNS (natalizumab, licensed drug) [41], depletion of lymphocytes and monocytes (alemtuzumab, phase 2 study) [42], or inhibition of lymphocyte egress from secondary lymphoid organs (fingolimod, phase 2 study) [43]], it can be postulated that any treatment reducing the presence of cytotoxic lymphocytes in the CNS would prevent the detrimental effects of an antiviral immune response at the brain tissue level. That B-cells exert a key role in MS has been definitively ascertained with the use of B-cell targeting drugs. Rituximab, a B-cell-depleting anti-CD20 monoclonal antibody, has been shown to reduce magnetic resonance imaging and clinical MS disease activity [44••]. The fact that rituximab is effective in EBV-induced lymphoproliferative disorders [45] suggests that this drug could ameliorate MS through elimination of EBV-infected B-cells.

## Conclusion

Accumulating evidence supports a role for EBV in MS development but whether this role is causative has yet to be determined. At the biological level, there is compatibility between: the ability of EBV to manipulate B-cell differentiation and activate cytotoxic T-cell responses; the main features of MS (intrathecal B-cell abnormalities and CD8+ T-cell activation); the mechanisms of action of immunosuppressive drugs targeting whole lymphocytes but also B-cells alone; and genetic variants that have been conclusively associated with MS. However, the association between EBV and MS goes beyond the biological level and includes established epidemiological and clinical features of the disease. From whatever perspective – biological, epidemiological or clinical – the contribution of EBV to MS pathogenesis is complex and a causative role for this virus appears plausible (Table 1). A better understanding of EBV involvement in MS will require the delineation of the nature of the interactions between genetic determinants of MS risk, other infectious and noninfectious environmental factors and, possibly, stochastic events that may favor EBV dysregulation. Elucidation of these aspects will have conceptual, diagnostic and therapeutic implications for MS.

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